

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

TURUN PATENTTITOIMISTO OY
P.O. Box 99
FIN-20521 Turku
FINLANDE

Date of mailing (day/month/year) 11 February 2002 (11.02.02)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference AP100089	
International application No. PCT/FI00/00730	International filing date (day/month/year) 29 August 2000 (29.08.00)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address BIOXID OY P.O. Box 114 FIN-20521 Turku Finland	State of Nationality FI	State of Residence FI
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address BIOXID OY Tykistökatu 4D, 4. krs FIN-20520 Turku Finland	State of Nationality FI	State of Residence FI
	Telephone No.	
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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Anne KARKACHI Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 09 May 2001 (09.05.01)	
International application No. PCT/FI00/00730	Applicant's or agent's file reference AP100089
International filing date (day/month/year) 29 August 2000 (29.08.00)	Priority date (day/month/year) 01 September 1999 (01.09.99)
Applicant AHOLA, Manja et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 28 February 2001 (28.02.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Charlotte ENGER
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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference AP100089	<div style="display: flex; justify-content: space-between;"> FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) </div>	
International application No. PCT/FI00/00730	International filing date (<i>day/month/year</i>) 29.08.2000	Priority date (<i>day/month/year</i>) 01.09.1999
International Patent Classification (IPC) or national classification and IPC ₇ A61L 27/00, A61L 27/54, A61L 31/00, A61L 31/16		
Applicant BIOXID OY et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 28.02.2001	Date of completion of this report 11.12.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer Barbro Nilsson/Els Telephone No. 08-782 25 00

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/FI00/00730

I. Basis of the report**1. With regard to the elements of the international application:***

- ☒ the international application as originally filed
- ☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____
- ☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____
- ☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____
- ☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI00/00730

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	<u>2-17</u>	YES
	Claims	<u>1</u>	NO
Inventive step (IS)	Claims		YES
	Claims	<u>2-17</u>	NO
Industrial applicability (IA)	Claims	<u>1-17</u>	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

US 5820917 (document D1) discloses a stent having two coating layers. The stent could be made of biostable or bioabsorbable polymers such as poly(lactic acid), refer to column 4, lines 32-34. The stent is provided with a heparin coating and the heparin coating with an overlayer of a polymer. The polymer is, e.g. poly(lactic acid) or cellulose, refer to claims 1, 5 and 7.

In EP 0604022 (document D2), a biodegradable stent is disclosed. The stent has a layer of, e.g. poly-L-lactic acid and a third layer is joined to the first layer and selected to release a therapeutic drug at a selected rate therefrom, refer to claim 7 and column 4, lines 25-27. Heparin or prostacyclin are appropriate drugs, refer to 43-44.

Therefore, the invention claimed in claim 1 is known from documents D1 and D2 and is thus not novel.

Moreover, US 5876433 discloses a stent which is coated with a plurality of layers of variable density of heparin coatings. US 5830480 discloses a silica-based bioactive glass from a sol-gel and a method for increasing the biocompatibility of the silica-based glass. The silica-based glass has an outer layer, which comprises biologically active molecules, for instance a growth factor.

.../...

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

In view of what is known from the cited documents above, the subject matter disclosed in claims 2-17 is considered obvious to a person skilled in the art.

In accordance with the arguments above, the invention in claim 1 is not novel and that in claims 2-17 is not considered to involve an inventive step. However, it is considered to be industrially applicable.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 March 2001 (08.03.2001)

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- (51) International Patent Classification⁷: **A61L 27/00**, 27/54, 31/00, 31/16 (74) Agent: **TURUN PATENTTITOIMISTO OY**; P.O. Box 99, FIN-20521 Turku (FI).
- (21) International Application Number: **PCT/FI00/00730** (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
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19991852 1 September 1999 (01.09.1999) FI (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
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Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **NOVEL MULTILAYERED MATERIAL BEARING A BIOLOGICALLY ACTIVE AGENT AND THE PREPARATION THEREOF**

(57) Abstract: The invention provides a material for medical use in humans and/or animals bearing a biologically active agent, said material being multilayered, as well as a device of this material and a method to produce it. The material comprises a core material, wherein said core material is formed into a body, optionally into a body having the shape of a finished device; two or more layers of coating material of which the first layer has been applied onto said core material and additional layers have been applied onto said coating material of a preceding layer; and wherein at least one of the layers comprise said biologically active agent. Characteristic for this material is that the coating material is a biopolymer, a sol-gel produced silica gel or a biologically active molecule.

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NOVEL MULTILAYERED MATERIAL BEARING A BIOLOGICALLY ACTIVE AGENT AND THE PREPARATION THEREOF

This invention relates to a novel multilayered material for medical use in humans and animals, in the form of a shaped body, that can optionally have an anatomic
5 and/or physiologic function, said multilayered material bearing biologically active agents. Furthermore, the invention concerns multilayered materials shaped into a body, into which such biologically active agents can be introduced. Still further, the invention concerns a method for preparing the novel multilayered material bearing biologically active agents.

10 BACKGROUND

Medical use of multilayered materials has been disclosed e.g. to provide sustained release or controlled release of oral or transdermal drugs (e.g. US-patents 4 451 260, 5 093 200, 5 645 858, 5 662 935, 5 681 583 and 5 332 577), or synthetic skin etc. (e.g. US-patents 4 060 081, 5 658 582 and 5 876 742).

15 Biodegradable polymers (Pitt et al. 1979, Ye and Chien 1996) as well as sol-gel processed silica xerogels (Kortesuo et al. 1999) have been used in controlled drug delivery devices, but their composites have not been used in these applications. Bioactive glass has been utilized in many orthopedic applications (Heikkilä et al. 1995). It is known that bioactive glass promotes bone formation.

20 It has not been possible to build up devices with elegant drug delivery properties with disclosed methods and materials. The novel combination of e.g. the above mentioned biodegradable materials in multilayered materials makes it possible e.g. to alter delivery properties by using different coating and/or core materials.

Multilayered materials enable the incorporation of biologically active agents into bioactive glass. This has been possible only by adsorption, which process is difficult to control.

OBJECTS OF THE INVENTION

- 5 One object of this invention is to provide medical devices made of a material tailored to be used in the human or animal body that is multilayered wherein desired regions and/or layers, i.e. coatings, of the device are provided with one or more agents having a desired biological activity.

Another object is to achieve a medical device having coatings into which a
10 therapeutically active agent is loaded, and from which said therapeutically active agent is released at a controlled rate.

SUMMARY OF THE INVENTION

Thus, according to one aspect, this invention concerns a material for medical use in humans and/or animals bearing a biologically active agent, said material being
15 multilayered comprising

- a) a core material, wherein said core material is formed into a body, optionally into a body having the shape of a finished device,
- b) two or more layers of coating material of which the first layer has been applied onto said core material and additional layers have been applied onto
20 said coating material of a preceding layer and
- c) said biologically active agent incorporated in at least one of the coating layers.

The coating material is a biopolymer, a sol-gel produced silica gel or a biologically active molecule.

According to another aspect, this invention concerns a device made of a material, useful for finishing into a device of a material for medical use in humans and/or
5 animals bearing a biologically active agent, wherein said material is multilayered and formed into a body of the shape of a finished device comprising

- a) a core material, wherein said core material is formed into a body, optionally into a body having the shape of a finished device,
- 10 b) two or more layers of coating material of which the first layer has been applied onto said core material and additional layers have been applied onto said coating material of a preceding layer and
- c) said biologically active agent incorporated in at least one of the coating layers.

The coating material is a biopolymer, a sol-gel produced silica gel or a biologically
15 active molecule.

According to a further aspect, this invention concerns a method for the preparation of a multilayered material for medical use in humans and/or animals bearing a biologically active agent, said material comprising

- 20 a) a core material, wherein said core material is formed into a body, optionally into a body having the shape of a finished device,
- b) two or more layers of coating material of which the first layer has been applied onto said core material and additional layers have been applied onto said coating material of a preceding layer and
- 25 c) said biologically active agent incorporated in at least one of the coating layers, and

wherein said coating material is a biopolymer, a sol-gel produced silica gel or a biologically active molecule. The method comprises the repeated steps of

- i) coating said core material or a coating material of a preceding layer with a coating material which optionally can comprise a biologically active agent and
- 5 ii) optionally binding a biologically active agent to the said coating.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic cross-sectional view of a rod of multilayered material.

Figure 2 shows a schematic cross-sectional view of a capsule of a multilayered material.

10 DETAILED DESCRIPTION OF THE INVENTION

According to invention the multilayered material bearing the biologically active agent has been shaped to a device. Said device comprises a core material formed into a body, optionally into a body having the shape of a finished device, wherein the biologically active agent is bound to the core material or to a coating material.

- 15 Said core material and the coating materials of the different layers can be the same or different and said biologically active agents comprised in, or bound to said core material or said coating material can as well be the same or different.

Definitions and preferred embodiments

- 20 The term "biologically active agent" shall be understood as an agent causing a valuable effect *in vivo*, such as a bioactive effect (i.e. promoting the binding of bone

to an artificial implant inserted into the mammal body), a therapeutic effect, or the like.

It shall be noted that certain biologically active agents also possess the ability to bind other biologically active agents. As an example can be mentioned silica gel, which as such may or may not, depending on e.g. its dissolution rate, nanoporosity and specific area, be biologically active (i.e. bioactive) in that it promotes tissue maintenance or formation. Furthermore, silica gel can be used as a matrix which can be loaded with a biologically active agent (e.g. a drug), which then is released in certain conditions, e.g. in body fluids. Silica gel can therefore be used for the controlled release of therapeutically active agents. Analogously polymers can act as vehicles used to release biologically active molecules. The function of polymers can be modified by molecular size and derivatization.

As particularly useful biologically active agents in implantable devices can be mentioned non-toxic inorganic molecules or polymers thereof; a silica gel as such or a silica gel loaded with a therapeutically active agent.

The biologically active agent can be any organic, inorganic or living agent that is biologically active. The biologically active agent can be a protein, a polypeptide, a polysaccharide (e.g. heparin), an oligosaccharide, a mono- or disaccharide, an organic compound, an organometallic compound or an inorganic compound containing any element with an atomic number ranging from 3 to 84. It can be a living or dead cell, bacterium, a virus or a part thereof. It can be a biologically active molecule such as a hormone, a growth factor, a growth factor producing virus, a growth factor inhibitor, a growth factor receptor, an integrin blocker (e.g. a IIa/IIIb inhibitor) or a complete or partial functional gene in sense or antisense orientation in a suitable expression vector or in any other expression vector construct for local delivery of therapeutically active agents. Biologically active agents include those especially useful for long-term therapy, such as hormonal treatment, for example

contraception and hormone replacement therapy, and for treatment of diseases such as osteoporosis, cancer, epilepsy, Parkinson's disease and pain. The suitable biologically active agents may be, e.g. anti-inflammatory agents, anti-infective (e.g. antibiotics and antiviral agents), analgesics and analgesic combinations, antiasthmatic agents, anticonvulsants, antidepressants, antidiabetic agents, antineoplastics, anticancer agents, antipsychotics, agents used for cardiovascular diseases. The multilayered material can be tailored to release the biologically active agent or agents composed in it at a controlled rate in *in vivo* conditions.

The word "body" shall be understood to be any defined piece or continuous article such as a granule, spherulite, sheet, film, plate, stick, pin, screw, tube, fiber, hollow fiber, woven fabric or non-woven fabric, or the like also when built to resemble human or animal body parts such as ear, nose, joints, filler in plastic form, etc. or parts thereof.

The term "biopolymer" shall be understood to mean either polymers based on renewable raw materials, biodegradable or not, e.g. cellulose, or synthetic polymers which are biodegradable, e.g. polylactides.

According to a preferred embodiment, the multilayered material bears more than one biologically active agent. The different biologically active agents can be composed in the same or different coating layers or in the core material.

According to another preferred embodiment each biologically active agent can be incorporated in the core material and/or a preferred coating layer or preferred layers. Further the biologically active agent can be designed to be composed in a desired region of the core or any coating layer of the body.

According to yet another preferred embodiment of this invention, the multilayered material is formed to a device to be implanted into the human or animal body to

serve different clinical applications, [e.g. stents, implants (dental or orthopedic), implants for controlled drug delivery, bone fixation pins, fixation plates, fixation bolts, regeneration matrixes, etc.]. The biologically active agents must of course be pharmaceutically acceptable.

- 5 According to a particularly valuable embodiment, the multilayered material is formed to a stent. A stent is a splint or tube to be placed temporarily or permanently inside a duct, canal or blood vessel to aid healing or relive an obstruction. Stents are inserted in blood vessels, e.g. in balloon angioplasty, where the balloon is placed inside an expandable stent (tube) which is expanded as the balloon is pressurized.
- 10 Known stents are typically tubes of metallic networks.

The stent according to this invention can be made of a biodegradable multilayered material or for example so that only the core and/or specific layers of said material are of a biodegradable material whereas the rest of the stent is of inert material. It can, moreover, be of a biodegradable bioactive glass fiber or of a sol-gel produced

15 silica gel fiber. The fiber is formed by multilayer coating according to this invention. Selected layers e.g. the inner wall of the tube can be coated with a silica gel loaded with a biologically active agent e.g. the anticoagulant drug heparin. The silica gel can e.g. be strongly bound to the inner wall of the tube via cleaved alkoxysilyl substituents on grafted monomers. A silica gel coating made in this

20 manner is stable and also thin, which is an important feature in this field of use. The outer wall of the tube can, if desired, be provided with other biologically active agents.

According to a particularly valuable embodiment the core of the multilayered system can be either a biodegradable silica body, e.g. bioactive glass or sol-gel

25 produced silica gel, or a biopolymer, which can be either biodegradable, e.g. polylactide, or inert, e.g. cellulose.

In the embodiment shown in figure 1 the core material 1 is bioactive glass or sol-gel produced silica gel coated with the following layers: a biodegradable polymer layer 2, a sol-gel produced silica gel or a biologically active molecule (e.g. heparin) layer 3 and another biodegradable polymer layer 4 of the same or a different polymer from the previous polymer layer 2. This arrangement, enables directed, targeted delivery of biologically active agents. The biologically active agents can be composed in both the sol-gel produced body (see example 1) or coating layer and the polymer body or coating layer (see example 2). The attachment of different layers can be improved by using different surface modification techniques, e.g. radiation induced grafting (example 2) or silylation treatment (example 3). All layers can have a different biologically active agent, if necessary. The thickness of the layers, e.g. layers 2, 3 and 4 in figure 1, can be varied widely, e.g. from about 100 nm to 1 mm depending on specific needs.

The embodiment shown schematically in figure 2 exemplifies that cumulative layers 2', 3', 4', 5' applied on the core material 1' can be tailored so as to cover only specific parts of the embodiment, in this case a capsule, but could be any device e.g. to be implanted into the human or animal body. Thus it is possible to tailor specific embodiments for specific purposes. The different layers at different locations can serve different purposes. For example capsules to be taken orally, capsules thus passing through the intestine, can be activated and/or dissolved by different pH and digestive enzymes at different locations of the intestine and activation and/or dissolution rate can be influenced by the chemical composition and area of each specific layer. Alternatively devices to be implanted can comprise asymmetrical layers with different compositions affecting different directions differently and/or layers releasing their biologically active agents at different stages of the lifecycle of the device e.g. triggered by a change in pH caused e.g. by a gradual decrease of an initial inflammation at the location of the implant.

A medical device manufactured according to this invention can be used for drug delivery. The degradation of the drug containing implant may or may not be a pH dependent process. The invention can be used to obtain targeted drug delivery. Functional devices that deliver drugs can be made by choice of core and coating material to disappear or not to disappear from the body. A medical device according to this invention can be used as a bone graft substitute to enhance the rate and probability of fracture healing by delivering antibiotics either alone or in addition to growth factors. In fractures involving, for example, the spine, the incorporation of anti-inflammatory agents and analgesics will help to control inflammation, which might delay the healing process, and thus contribute to patient comfort during the healing process.

Local drug release after cancer surgery using a medical device according to this invention could provide targeted and long-lasting disease control.

Inducing the growth of blood vessels in bioartificial tissues (angiogenesis) could be stimulated by coating a medical device according to this invention with growth factors or growth factor producing viruses, which growth factors trigger blood vessel formation.

A woven or nonwoven mat made of fibers according to this invention containing one or more biologically active agents can be used for a temporary skin substitute or for guided tissue regeneration.

A multilayered body according to this invention can be designed into a pharmaceutical for oral use optionally comprising multiple drugs. Multiple layers of specific materials could be used to e.g. achieve slow release of the drug or drugs and/or it could be used to e.g. ensure controlled release of the drug or drugs in e.g. a part of the digestive system, which is optimal for the uptake of each specific drug.

The main field of this invention can briefly be summarized as utilization of novel multilayered materials equipped with a biologically active agent which give raise to a desired respond when brought into contact with living tissues giving said materials tailored properties for medical use in humans and animals.

- 5 The invention is disclosed in more detail by the following experiments.

EXPERIMENTAL SECTION

Example 1

Application of heparin loaded silica gel onto a grafted PLLA-co-CL copolymer

- 10 In this example, heparin is immobilized onto silica gel and then grafted PLLA (poly-L-lactide) sheets are coated with the heparinized silica-gel. Bulk heparinized silica-gel samples were obtained for the drug delivery ability tests. It is known that silica-gel can be used as a drug delivery system (Kortesuo et al. 1999).

Preparation of silica-sol

- 15 The heparin immobilized silica-sol was prepared by a two step sol-gel process using acid as a catalyst (Brinker and Scherer 1990, Ellerby et al. 1992). The following reagents were used: tetraethoxysilane (TEOS) (Aldrich), deionized water, ethanol nitric acid (HNO₃) (Merck) and ammonium hydroxide (NH₄OH). The r-value (water/TEOS molar ratio) was 3.55. Ethanol was used as a solvent to obtain better viscosity (water/ethanol molar ratio = 1). NH₄OH was used to raise pH up to 4.5.
- 20 Heparin used was a sodium salt of heparin obtained from Orion Corporation (biological activity 139 I.U./mg). To obtain 100 ml hydrolysis solution, 48 g of

tetraethoxysilane (TEOS), 45 g of deionized water, 4 g of glycerol and 10.1 g of catalyst (0.04 M HNO₃) were added to a glass container and stirred until the inorganic water phase and the organic TEOS phase had become homogenized.

To obtain small bulk pieces for the drug delivery test, the hydrolysis solution was divided into smaller samples ($V = 0.5$ ml) that were aged in sealed polystyrene container at 40 °C and 40 % relative humidity for 3 days. After this gelation process samples were dried at room temperature for an additional 3 days. The heparin content in one gel piece varied from one to 15 weight-% (calculated from the dry weight). The H₂O/TEOS-molaratio (r-value) was between 14 and 16, and pH was 4.5. The same sol was used when the grafted polymers were coated.

The coating was applied to Poly(L-lactide)-co-ε-caprolactone (PLLA-co-CL) sheets immediately after hydrolysis by dipping technique. The uniformity of coating was examined with scanning electron microscopy (SEM) measurements.

Functionalization of the polymer

PLLA and PCL (polycaprolactone) were polymerized according to a method described by Holmlund (Holmlund 1999). The pre-weighed polymer samples were irradiated by using an Electrocurtain[®] electron accelerator in air at an acceleration voltage of 175 kV (Södergård 1998 a and b). The irradiated films were removed from the accelerator and immersed into the monomer solutions at ambient temperature for various reaction times without using any homopolymerization inhibitor.

Polymer:	Poly(L-lactide)-co-ε-caprolactone (PLLA-co-CL)
Monomer:	Acrylamide (Promega)
Extent of grafting:	About 20 %
Monomer distribution:	As a uniform coating

The extent of grafting was gravimetrically determined from the following equation:

$$E (\%) = 100 \cdot \frac{m_1 - m_0}{m_0}$$

where m_0 and m_1 are the weights of the ungrafted and the grafted sample, respectively.

- 5 The monomer solutions were purged with nitrogen for at least 30 minutes before the grafting in order to minimize the presence of oxygen during the grafting process. The grafted films were washed with ion-exchanged water for several hours in order to remove homopolymer, and dried to constant weight in vacuum at room temperature.

10 Tests

The following functional tests were performed: the biocompatibility of the materials was examined by using cultured cells, the release of heparin and silica were studied by dissolution testing, and the biological activity of the bound and released heparin was determined by a thrombin assay (Kang et al. 1997). From the dissolution test
15 heparin was determined by a toluidine blue test (Smith et al. 1980 and Park et al. 1991) and silica by a spectroscopic method (Koch and Koch-Dedic 1974). Scanning electron microscopy (SEM) was used to study the morphological characteristics of the silica-gel coating. Materials were *in vitro* tested by culturing cells on materials and by measuring cytotoxicity of materials.

20 Dissolution test

SBF was prepared by dissolving NaCl, NaHCO₃, KCl, K₂HPO₄ × 3H₂O, MgCl₂ × 6H₂O, CaCl₂, Na₂SO₄, TRIZMA[®] HCl and TRIZMA[®] base as shown in Table 1.

The fluid was adjusted at physiological pH 7.40 and temperature 37 °C. The composition of inorganic ions emulated that of human blood plasma.

Table 1. Reagents used for the SBF solution.

Reagent	Amount/1 dm ³ (H ₂ O)	Manufacture/purity
NaCl	7.9951	Riedel-deHaën, pro analysis/ 99.8%
NaHCO ₃	0.3534	Merck, pro analysis/99.5 %
KCl	0.2243	Merck, pro analysis/99.5 %
K ₂ HPO ₄ · 3H ₂ O	0.2281	Merck, pro analysis/99 %
MgCl ₂ · 6H ₂ O	0.3053	Merck, pro analysis/99 %
CaCl ₂	0.2776	Merck, pro analysis/99 %
Na ₂ SO ₄	0.0709	Merck, pro analysis/99 %
TRIZMA HCl *	6.2414	Sigma Ultra/ 99.9 %
TRIZMA base **	1.2591	Sigma Ultra/99.9 %

* TRIZMA[®] HCl: tris[hydroxymethyl]aminomethane hydrochloride (HOCH₂)₃CNH₂ · HCl

5 ** TRIZMA[®] base: tris[hydroxymethyl]aminomethane (HOCH₂)₃CNH₂

From 20 to 25 mg of heparinized silica gel was immersed in 50 ml SBF in a polyethylene bottle covered with a tight lid. Two parallel samples and six different time points were used. All bottles were placed in a shaking water bath at 37 °C. All samples were filtered before the ion concentration (Si) analysis was carried out by spectroscopic method described by Boltz and Mellon 1947.

Cell growth and cytotoxicity testing

The following materials were tested: copolymer as such, PLLA, CL, copolymer grafted in both ways and bulk heparinized silica gel. The materials were *in vitro* tested by culturing cells on materials and by measuring cytotoxicity of materials.

15 The purpose of this was to evaluate whether the grafting process and grafted materials are safe for living tissues, and also to choose the best candidates for *in*

vivo testing. Human gingival fibroblasts (Häkkinen 1995) were routinely cultured in Dulbecco's Modification of Eagle's Medium (DMEM), including 10 % (v/v) Foetal Calf Serum (FCS, kibbutz Beit Haemek, Israel), 4,500 mg/l glucose, 3.7 g/l NaHCO₃ and penicillin-streptomycin solution (GibcoBRL, 10,000 U/ml and 10,000
5 µg/ml in saline) 1 ml/l. Cells were cultured on petridishes (Ø 10 cm) at +37 °C and 5 % CO₂ atmosphere. The medium was changed every other day and the cells were harvested at confluency. Only cells from nearly confluent dishes were used for experiments.

Materials used for cell culturing were washed with 20 % ethanol and sterile
10 deionized water. Then they were attached to culture dishes with silicone grease. Amount of cells per sample material was about 1/6 of the confluent petridish. Medium was changed every other day and cell growth was followed and investigated with a microscope.

Cytotoxicity of materials was evaluated using a modification of the lactate
15 dehydrogenase (LDH) method (Korzeniewski and Callewaert 1983). Materials were tested as extracts. Standard 6-well plates (Nunc) were used. One confluent petridish (Ø 10 cm) was used per test. Cells were first washed with 4 ml of EDTA solution [in phosphate buffer solution (PBS), pH 7.4] and then incubated 5 minutes in 4 ml of trypsin EDTA solution (40/0.4) at +37 °C. [Trypsin stock = 2.5 % (w/v) in normal
20 saline, GibcoBRL]. Detached cells were transferred into a 15 ml centrifuge tube and centrifuged 5 minutes at 800 rpm. Finally cells were suspended in 5 ml of medium. From this suspension 200 µl per well was added. Cells were cultured in these plates as mentioned before using 2 ml medium per well, changing medium every other day until the cultures had reached confluency.

25 When all cultures had almost reached confluency, the test materials were extracted. Materials (ca. 0.5 cm²) were dipped into 20 % ethanol and rinsed with sterile deionized water. Then they were dipped into sterile eppendorf tubes and 1 ml of

medium was added. These tubes were incubated for 24 hours at +37 °C. After that the medium in the wells of the test plates was removed and dead cells detached with 0.02 weight-% EDTA solution. Then 1 ml of fresh medium was added into each well. The extracts were added, and into wells for spontaneous LDH release 1 ml of medium was added. For wells of maximal LDH release 200 µl of 10 % Triton X-100 and 1 ml of medium was added. This test system was incubated in cell culture conditions (as mentioned before) for 24 hours.

After 24 hours a sample of 500 µl from each well was taken. LDH measurement was carried out at room temperature (20—25 °C). Solution A was prepared by pipetting 432 µl of 30 mM sodiumpyruvate (Sigma) in Tris-Cl buffer (TRIZMA®, Sigma), pH 7.4 and 432 µl of 6.6 mM NADH (FlukaChem) in the same buffer. The reagent was then diluted with 2550 µl of the same buffer. The ready made solution A was kept in dark between single measurements and vortexed carefully before use.

The samples were diluted by mixing 200 µl of the sample with 700 µl of Tris-buffer (same as above). They were vortexed and transferred into cuvettes. Measurements were carried out using a Shimadzu UV/Vis spectrophotometer at 340 nm wavelength. The reaction was started by adding 100 µl of solution A into the cuvette containing a sample, and the reaction was followed for 7 minutes. The slope was measured for the first 120 second period and used for indication of the amount of LDH released into the medium. Cytotoxicity (%D) was calculated by using the equation:

$$\%D = \left[\frac{(|A| - |B|)}{(|C| - |B|)} \right] \cdot 100 \%$$

A = sample slope

B = spontaneous release well slope and

C = Triton X-100 well slope.

Results

- 5 Both the dissolution rate of the silica-gel and releasing rate of heparin was examined by using the bulk gel prepared by sol-gel technique. Up to 15 %, calculated from the theoretical dry weight, of heparin was successfully immobilized to the silica-gel produced by an acid catalyzed hydrolysis reaction.

During the 24 days, 45 % of heparin loaded was released. Each heparin
10 concentration used, 1–15 mass-% calculated from the theoretical dry weight, had similar releasing profile. Heparin concentrations were studied by toluidine blue method (Smith et al. 1980 and Park et al. 1991). Heparin released retained its biological activity as an anticoagulant when examined by the HEPRN[®] method.

According to information obtained from SEM studies, a uniform silica-gel coating
15 was obtained on a surface of the acrylamide grafted PLLA-co-CL sheet. The thickness of the coating produced was 0.3 mm and its cracking after bending was minimal.

When human gingival fibroblasts were grown under cell culture conditions on coverslips, without and together with small silica-gel particles, it was found that cell
20 growth was not influenced by the presence of the gel. Cells divided and spread normally and finally covered the silica-gel particles even though they were topographically elevated from the substratum surface. From the results of the *in vitro* test it seems obvious that silica-gels do not have any toxic or other harmful effects on fibroblasts growing in contact with the material. The present results agree
25 with previous results indicating that silica-gels are biocompatible materials and

promising dissolvable vehicles for delivery of biologically active molecules. The gels have no harmful effects on living tissues as can also be seen from the results of *in vivo* testing (Ahola et al., 1997 and Kortesoja et al., 1999).

Example 2

- 5 Control of the retention of a biologically active molecule (heparin) to a polymer carrier by varying the chemical reactivity (functionality) of the carrier by grafting.

In this example, the degradable polymer (PLLA) is coated with biologically active agents. The surface properties of the copolymers of poly-L-lactide (PLLA) (Neste Ltd.) and poly-ε-caprolactone (PCL) (Sigma Co.) (PLLA/PCL = 50/50) were altered
10 by grafting with acrylamide (Promega Co.) onto the surface of PLLA-co-CL by electron beam (EB) irradiation (Holmlund 1999). The grafted polymer sheets were washed thoroughly with ethanol and deionized water. The grafted surface was then able to attach silica-gel or biologically active molecules such as heparin.

The grafted polymer was allowed to react with heparin solution (0.025 mg
15 heparin/5 ml incubation solution). Sodium salt of heparin was from Orion Pharma (biological activity 139 I.U./mg). pH of the solution was adjusted before heparin addition, by acetic acid buffer (pH 4.5) and TRIS- or phosphate buffer (pH 8). The polymer sheets were incubated with this heparin solution. The incubation times varied from 2 to 96 h and incubations were carried out at 25 or 37 °C. Sheets with
20 attached heparin were washed thoroughly with deionized water.

The silica-sol immobilized heparin was prepared by a two step sol-gel process using nitric acid as a catalyst (Ellerby et al. 1992). The following reagents were used, TEOS (Aldrich), deionized water, nitric acid (HNO₃) (Merck), ammonium hydroxide (NH₄OH) and heparin sodium salt (Orion, biological activity

139 I.U./mg). To obtain 100 ml hydrolysis solution 48 g of TEOS, 45 g of deionized water and 10.1 g of catalyst (0.04 M HNO₃) were added to a glass beaker and stirred until the ingredients formed a homogenous solution. The silica gel coating was applied by dipping grafted polymer sheets into the homogenous hydrolysis solution.

The following functional tests were performed: the biocompatibility of the materials was examined by using cultured cells, the release of heparin and silica were studied by dissolution test and biological activity of the bound and released heparin was determined by the thrombin assay (Kang et al. 1997). From the dissolution test heparin was studied by toluidine blue test (Smith et al. 1980 and Park et al. 1991) and silica by spectroscopic method (Koch and Koch-Dedic 1974). SEM was used to study the morphological characteristics of the silica gel coating.

Results and discussion

The results from the cell culture tests, cytotoxicity test (Korzeniewski and Callewaert 1983), suggest that acrylamide grafting does not alter the biocompatibility of the PLLA-co-CL. Both contact and extract tests were carried out, and no significant differences between these results were observed.

Direct immobilization of heparin

Changing the reaction conditions, e.g. reaction time or temperature and pH of the incubation solution, could vary the amount of heparin immobilized on the grafted PLLA-co-CL. The attachment of heparin was best when acidic conditions (pH 4.5, acetic acid) were used (Table 1). Up to 98 µg/cm² of heparin was immobilized on the surface of the PLLA-co-CL graft polymer. The results were rather good even when only deionized water was utilized as a solvent. If the incubation solution is basic, the chemical structure of the buffering solution must be taken into account.

TRIS-buffer, which is a combined solution of TRIZMA HCl: tris[hydroxymethyl]aminomethane hydrochloride $(\text{HOCH}_2)_3\text{CNH}_2 \times \text{HCl}$ (Sigma Ultra/ 99.9 %) and TRIZMA base: tris[hydroxymethyl]aminomethane $(\text{HOCH}_2)_3\text{CNH}_2$ (Sigma Ultra/ 99.9 %), should be avoid because TRIS may react with heparin preventing the attachment to the polymer. By using a phosphate buffer the attachment of heparin is possible, so the absence of heparin on the polymer when using TRIS is not a matter of pH alone. Overall, the attachment of heparin to the surface was better in acidic conditions than in basic.

Table 1

Effect of pH on the attachment of heparin on the surface of PLLA-co-CL polymer sheets. Size of the sheets used for the measurements was 0.25 cm^2

pH	pH adjusting reagent	Heparin (mg) (toluidine test*)	Heparin (μg)/ cm^2
4.5	acetic acid buffer	23 ± 5	94
5.7	deionized water	9 ± 2	36
8.0	TRIS-buffer	-	-
7.8	phosphate buffer	3 ± 1	10

* The result is a mean value of three parallel measurements.

According to the thrombin test, the immobilized heparin retains its biological activity against thrombin formation. At least 76 % of heparin, observed on the surface by the toluidine blue test, showed biological activity as well. In the dissolution test, one week in simulated body fluid at 37°C , heparin was still immobilized since no heparin was released as estimated by the toluidine blue test or by the thrombin test.

Silica gel immobilized heparin coating

SEM pictures of the grafted surfaces showed that a uniform, about 0.3 μm thick, silica-gel coating was obtained with the dipping technique. Cracking of the silica-gel layer was minimal after bending sheets several times 90°. Heparin released from the silica coatings during the dissolution test retained its anticoagulant activity. The releasing rate of heparin follows that observed for silica, after one week half of the immobilized heparin was released.

Example 3

The attachment of a polymeric layer on a top of bioactive glass.

- 10 In this example, the degradable bioactive glass-13 (composition: 6 % Na_2O , 12 % K_2O , 5 % MgO , 20 % CaO , 4 % P_2O_5 and 53 % Si_2O_2) (Brink 1997) is coated with biocompatible, degradable polycaprolactone polymer by using organomodified silanes as coupling agents.

Silylation of fibers

- 15 The biodegradable glass-13 fibers were prepared from glass melt (near 1100 °C) by drawing technique. After cooling down to room temperature the fibers were cut into small pieces (circa 10 cm long). These fibers were placed into a 50 ml falcon tube and the tube was filled with silylation reagent (2 % of dichlorodimethylsilane ($\text{C}_2\text{H}_6\text{Cl}_2\text{Si}$) in trichloromethane (CHCl_3) solution). The silylation lasted for 10 min.
- 20 Before washing the fibers carefully with deionized water, the silylation coating was let to stabilize for 24 h. The washed, silylated fibers were dried in a vacuum deccicator for an additional 24 h.

Polymer coating of fibers

The polycaprolactone (PCL) was dissolved in tetrahydrofurane (THF) (4,149 g of PCL in 100 ml of THF) and the silylated glass fibers were dipped into the solution. After dipping the fibers were dried at room temperature.

5 *Results and conclusion*

The polymer coating layer formed on the glass fiber was uniform and the method explained above is an easy way to modify glass fibers.

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CLAIMS

1. A material for medical use in humans and/or animals bearing a biologically active agent, said material being multilayered comprising
 - a) a core material, wherein said core material is formed into a body,
5 optionally into a body having the shape of a finished device,
 - b) two or more layers of coating material of which the first layer has been applied onto said core material and additional layers have been applied onto said coating material of a preceding layer and
 - c) said biologically active agent incorporated in at least one of the coating
10 layers,

characterized in that said coating material is a biopolymer, a sol-gel produced silica gel or a biologically active molecule.
2. The material according to claim 1 **characterized** in that the core material is a biodegradable silica body, e.g. bioactive glass or sol-gel produced silica gel, or a
15 biopolymer, e.g. a polylactide or a cellulose.
3. The material according to claim 1 or 2, **characterized** in that said coating material is a biopolymer, e.g. a polylactide or a cellulose.
4. The material according to claim 1 or 2, **characterized** in that said coating material is a sol-gel produced silica gel.
- 20 5. The material according to claim 1 or 2, **characterized** in that said coating material is heparin.

6. The material according to any of the claims 1 to 5, **characterized** in that a biologically active agent is composed in a sol-gel produced silica gel body or a sol-gel produced silica gel coating layer, or a biopolymer body or biopolymer coating layer.
- 5 7. The material according to any of the claims 1 to 6, **characterized** in that the core material and all coating materials are biodegradable.
8. The material according to any of the claims 1 to 7, **characterized** in that it is a body in the shape of a granule, spherulite, sheet, film, plate, stick, pin, screw, tube, fiber, hollow fiber, woven fabric or non-woven fabric or the like also when built to
10 resemble human or animal body parts such as ear, nose, joints etc. or parts thereof.
9. The material according to claim 8, **characterized** in that it is shaped to a stent, dental or orthopedic implant; implant for controlled drug delivery; bone fixation pin, fixation plate, regeneration matrix, or the like also when built to resemble human or animal body parts such as ear, nose, joints etc. or parts thereof.
- 15 10. The material according to any of the claims 1 to 9, **characterized** in that the biologically active agent is a polypeptide, a protein, a polysaccharide, an oligosaccharide, a mono- or disaccharide, an organic compound, an organometallic compound or an inorganic compound containing any element with an atomic number ranging from 3 to 84.
- 20 11. The material according to claim 10, **characterized** in that the biologically active agent is
- an inorganic ion or a polymer thereof,
 - silica gel as such or silica gel loaded with a therapeutical agent,
 - heparin or its derivative,
 - 25 - a growth factor,

- a growth factor producing virus,
- a growth factor inhibitor,
- an integrin blocker (e.g. a IIa/IIIb inhibitor)
- an oligonucleotide or
- 5 - a complete functional or partial gene in sense or antisense orientation in a suitable expression vector or any other expression vector construct

for local delivery of said biologically active agent.

12. The material according to claim 9, **characterized** in that it is shaped to a stent, the inner wall of which is provided with a biologically active agent; which is

- 10 - an inorganic ion or a polymer thereof,
- silica gel as such or silica gel loaded with a therapeutical agent,
- heparin,
- a growth factor,
- a growth factor producing virus,
- 15 - a growth factor inhibitor,
- an integrin blocker (e.g. a IIa/IIIb inhibitor),
- an oligonucleotide or
- a complete or partial functional gene in sense or antisense orientation in a suitable expression vector or any other expression vector construct; and

20 which biologically active agent is released at a controlled rate in *in vivo* conditions.

13. A device made of a material, useful for finishing into a device of a material for medical use in humans and/or animals, said material bearing or being capable of binding a biologically active agent, wherein said material is multilayered and formed into a body of the shape of a finished device comprising

- 25 a) a core material, wherein said core material is formed into a body, optionally into a body having the shape of a finished device,

b) two or more layers of coating material of which the first layer has been applied onto said core material and additional layers have been applied onto said coating material of a preceding layer and

5 c) at least one layer of coating material capable of binding a said biologically active agent

characterized in that said coating material is a biopolymer, a sol-gel produced silica gel or a biologically active molecule.

14. A method for the preparation of a multilayered material for medical use in humans and/or animals bearing a biologically active agent, said material comprising

10 a) a core material, wherein said core material is formed into a body, optionally into a body having the shape of a finished device,

b) two or more layers of coating material of which the first layer has been applied onto said core material and additional layers have been applied onto said coating material of a preceding layer and

15 c) said biologically active agent incorporated in at least one of the coating layers, and

wherein said coating material is a biopolymer, a sol-gel produced silica gel or a biologically active molecule, **characterized** by the repeated steps of

20 i) coating said core material or a coating material of a preceding layer with a coating material which optionally can comprise a biologically active agent and

ii) optionally binding a biologically active agent to the said coating.

15. The method according to claim 14 wherein the core material has been formed into a body, optionally into a body having the shape of a finished device, **characterized** in that a coating is generated on the core material or a coating material of a preceding layer on a desired region of the body.

25

16. The method according to claim 14 or 15, **characterized** in that the attachment of a coating layer is improved by using surface modification techniques of the surface to be coated, i.e. the core surface or the surface of the previous coating layer.
- 5 17. The method according to claim 16, **characterized** in that the surface modification technique used is radiation induced grafting or silylation treatment.

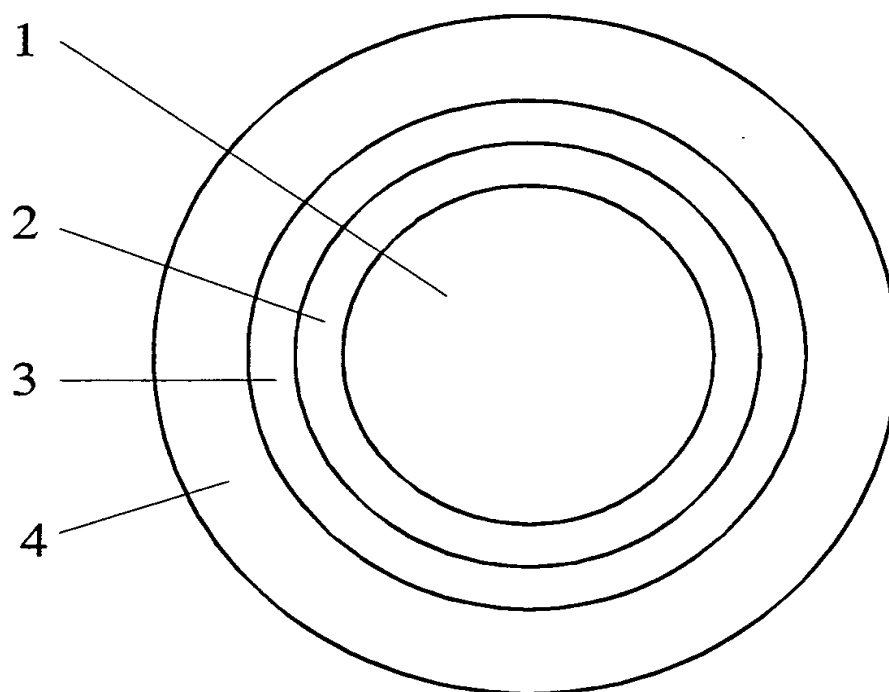


Figure 1

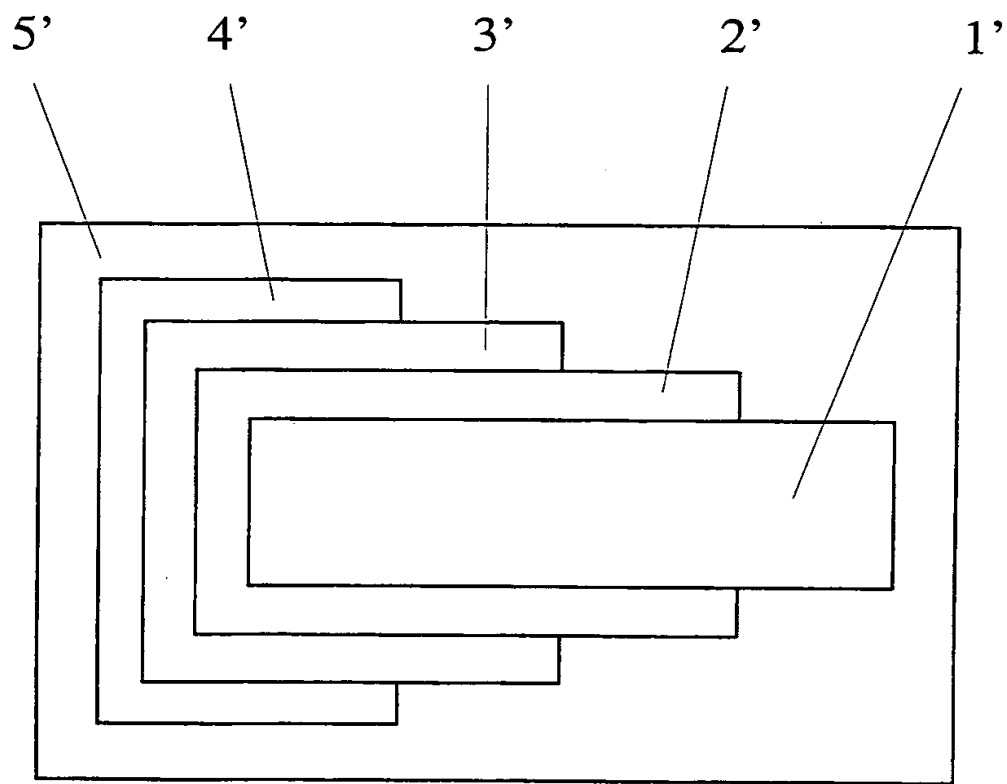


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 00/00730

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61L 27/00, A61L 27/54, A61L 31/00, A61L 31/16
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5820917 A (RONALD J. TUCH), 13 October 1998 (13.10.98), claims 1-9 --	1-17
X	US 5876433 A (ANTHONY C. LUNN), 2 March 1999 (02.03.99), claims 1-2 --	1-17
A	EP 0923953 A2 (SCHNEIDER (USA) INC.), 23 June 1999 (23.06.99), claims 1-9,21 --	1-17

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

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 "&" document member of the same patent family

Date of the actual completion of the international search

22 November 2000

Date of mailing of the international search report

29 -11- 2000

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 00/00730

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

02/11/00

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